27th ECCMID Vienna, Austria 22 – 25 April 2017 | ESCMID

ifik institut für infektionskrankheiten Ein Institut der Universität Bern
u b UNIVERSITÄT BERN

Vienna – April 22nd, 2017

(Hall B – 13:30-15:30)

Session SY021:

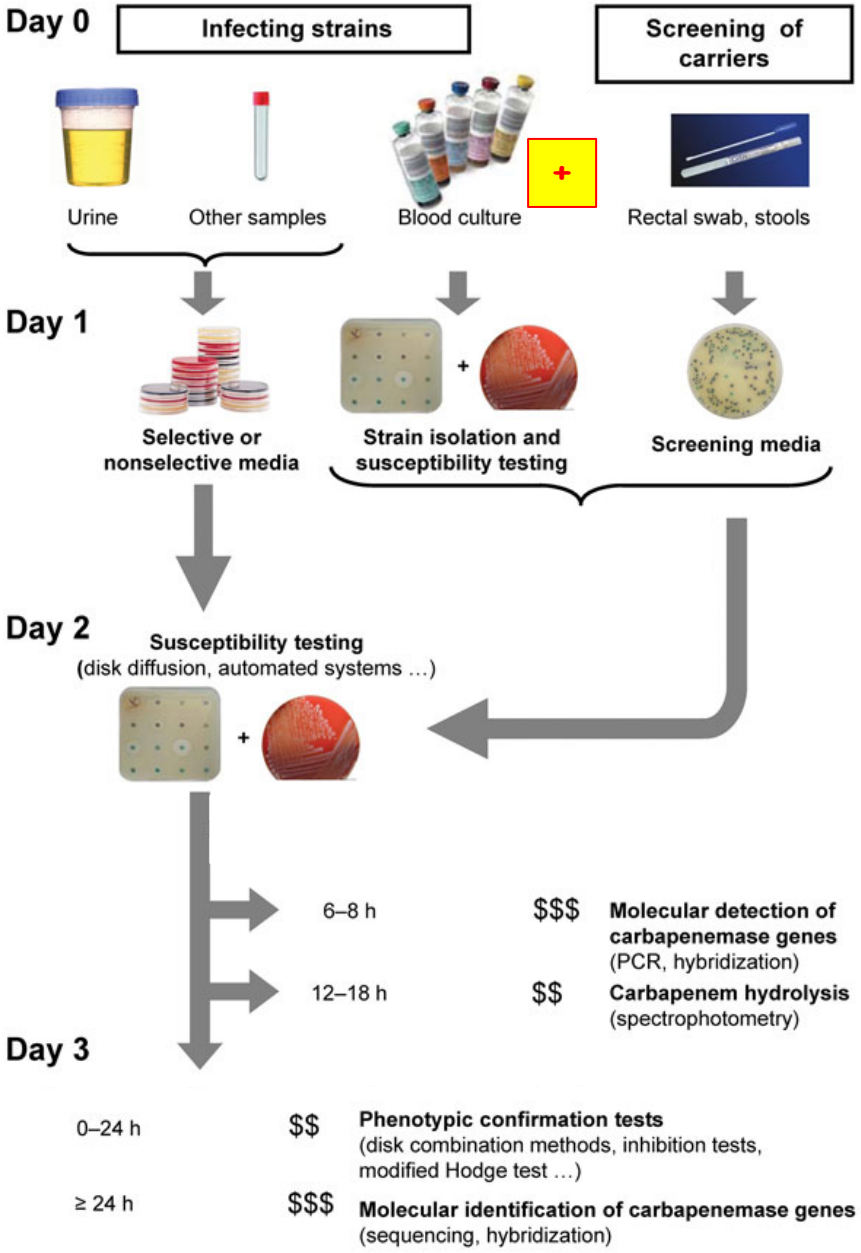
Reporting Antibiogram Data

Direct Reporting of Resistance Mechanisms

Prof. Andrea Endimiani, MD, PhD

Institute for Infectious Diseases (IFIK)

University of Bern, Switzerland



Adapted from Nordmann P. *et al.*, EID 18(9), Sept 2012

Outcome

Empirical treatment

Isolation

Directed treatment

Septic shock

Inappropriate antibiotics within the first 6 hours is associated with a 5-fold higher mortality risk

[Kumar A *et al.*, Chest, 2009]

BSIs

Mean decrease in survival of 7.6% for each hour after the onset of BSI until initiation of effective antibiotics

[Kumar A *et al.*, Crit Care Med, 2006]

MALDI-TOF MS

Multicenter evaluation of the Sepsityper™ extraction kit and MALDI-TOF MS for direct identification of positive blood culture isolates using the BD BACTEC™ FX and VersaTREK® diagnostic blood culture systems

Schieffer KM *et al.*, J Appl Micro; 116, 2014

Sepsityper extraction kit (Bruker Daltonics)

~15 min
(*vs.* 7-45 min for lab methods)

~80-85% efficient extraction
(*vs.* 85-90% for lab methods)

n=411 +BCs

Accuracy of Sepsityper extraction/MALDI-TOF MS (SE-MALDI) identification method

	Number of blood culture samples analysed (% correct)	
Organism group		Total
Staphylococci		171 (94·2)
Streptococci		15 (93·3)
Enterococci		25 (92·0)
Other Gram-positive bacteria		14 (64·3)
Enterobacteriaceae		88 (98·9)
Other Gram-negative bacilli		4 (100)
Anaerobic bacteria		2 (0·0)
Yeasts		6 (50·0)
Mixed organisms		33 (93·9)
Total	Incl. those with score <1.6	358 (92·7)

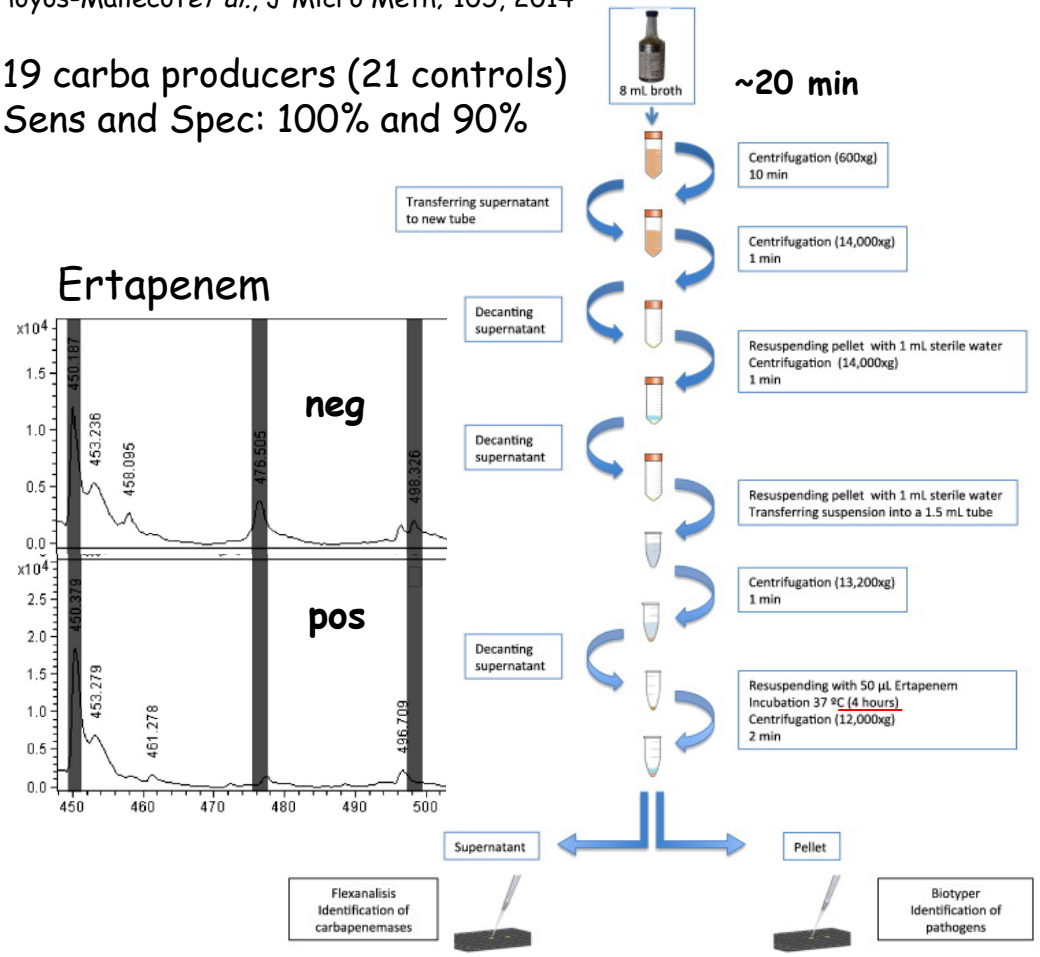
ID in ~1h
Reduction of 6-83 hrs

Rapid detection and identification of strains carrying carbapenemases directly from positive blood cultures using MALDI-TOF MS

Hoyos-Mallecot *et al.*, J Micro Meth; 105, 2014

19 carba producers (21 controls)
Sens and Spec: 100% and 90%

~20 min



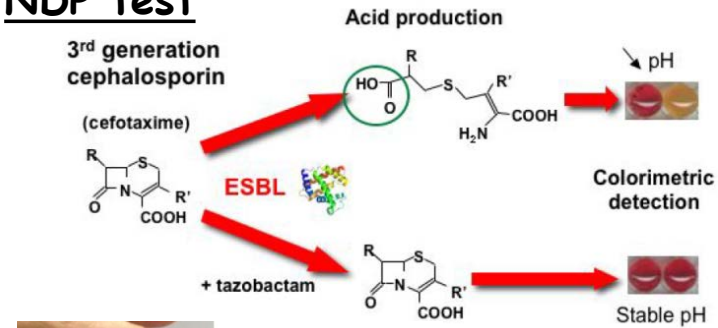
Generic activity in ~4.5 hrs
Can be reduced to ~30 min-2 hrs

Colorimetric tests

Rapid Detection of ESBL-Producing *Enterobacteriaceae* in Blood Cultures

Dortet L. *et al.*, Emerg Infect Dis; 21:3, 2015

NDP test



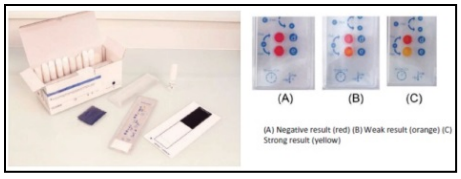
Overall, ~30 min
Sens/Spec: 100%

Rapid detection of carbapenemase-producing Enterobacteriaceae from blood cultures

Dortet L. *et al.*, Clin Microbiol Inf; 20, 2014

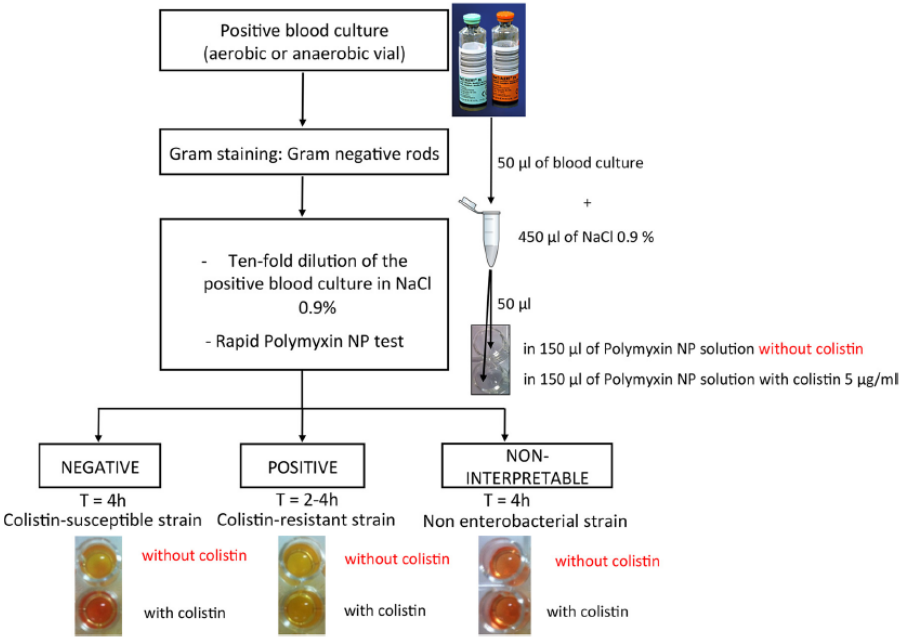
Preparation from +BCs: ~3 hrs
Reaction in 5 min-1 h (max 2 hrs)
Sens and Spec 98% and 100%

The RAPIDEC Carba NP ®Test



Rapid Detection of Polymyxin-Resistant *Enterobacteriaceae* from Blood Cultures

Jayol A. *et al.*, J Clin Micro; 54:9, 2016



Final results in 2-4 hrs
Sens and Spec: 98% and 100%

RAPID POLYMYXIN NP

~10 Euro

Gram-negatives

Antibiotics	Mechanism	Most frequent protein/genes
Cephalosporins	ESBLs	CTX-Ms
		TEMs and SHVs (not all are ESBLs)
		PERs, VEBs, GESs, SFO, etc.
	Plasmidic AmpCs	CMYs, DHAs
		FOXs, LATs, BILs, MOXs, MIRs, ACTs
	Chromos. AmpCs	Promoter region (*E. coli*)
		AmpD (*Enterobacter* spp.)
Carbapenems	Carbapenemases	Class A: - KPCs, GESs
		Class B: - NDMs, VIMs, IMPs
		- SPMs, GIMs, AIMs, SIMs
		Class D: - OXA-48
		- OXA-48-like
		- OXA-23, -24/40, -58
	Porins	OmpK36 (*K. pneumoniae)*
		OmpF/OmpC (*E. coli*)
		OmpF/OmpC (*E. cloacae*)
Quinolones	QRDR	Substitutions in GyrA and ParC
	PMQR determinants	QnrA/B/S, QepA, aac(6)-Ib-cr
Aminoglycosides	AMEs	aac(6)-I-like, ant(3)-like, aph(3)-like
	16S rRNA methyl.	ArmA, RmtA/B/C/D/E, NpmA
Polymyxins	Plasmid mediated	Mcr-1-/-2-like
	Chromosomal	*mgrB* (*K. pneumoniae*)
Fosfomycin	Plasmid-mediated	*fos* genes

Ideal system:

- Species identification
- Resistance mechanisms
- Kit and automated (easy to use)
- High sensitivity/specificity
- Clinical samples (or at least +BCs)
- Rapid
- Cost-effective

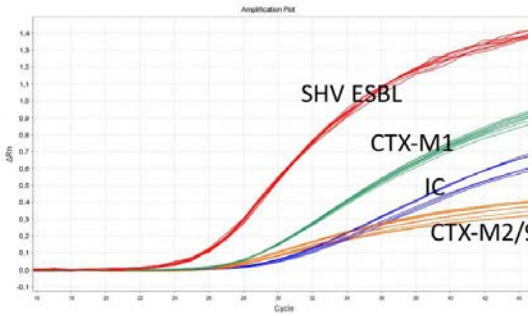
Gram-positives

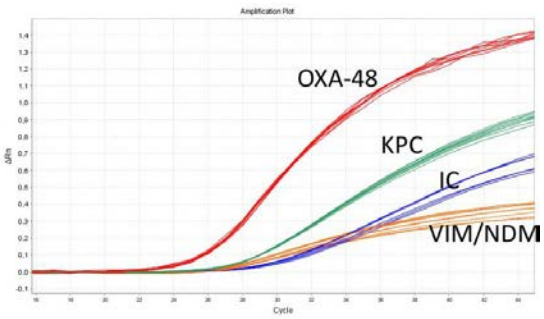
Antibiotics	Mechanism	Most frequent genes
B-lactams	PBP2	*mecA, mecAv, mecB, mecC*
	β-lactamases	*blaZ*
Glycopeptides		*vanA, vanB, vanC/D/E/G/Z*
Quinolones	QRDR	Substitutions in GyrA and ParC
Aminoglycosides	AMEs	*aac(6)-I-like, aph(2)-I-like…..*
Macrolides		*ermA/B/C, msr, mefA/E, ereA/B…*
Clindamycin		
Lincosamides		*lnuA-D, lsaB-E*
Streptogramins		*vat, vga, vgb* genes
Linezolid		*cfr*
Mupirocin		*mupR*
Phenicols		*cat* genes, *fexA*
Trimethoprim	Folate inhibitors	*dfrA, dfrD, dfrG, dfrK*
Tetracyclines		*tetK, tetL,* tetM/O/Q/S…….
Fusidic acid		*fusB, fusC, fusD*

Check-Direct Screening, Check-Points

Rectal swab
(Copan, ESwab)

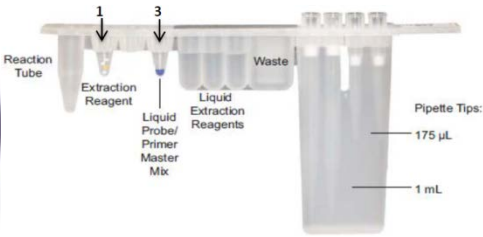
- Rapid preparation
- Time to results (**<3 hrs**)





BD MAX

Reagent strip



Check-Direct ESBL Screen for BD MAX™

CTX-M-1 group
CTX-M-2 group
CTX-M-9 group
SHV-ESBL

Check-Direct CPE Screen for BD MAX™

KPC
OXA-48-like
VIM
NDM

~30 Euro

Performance of the BD MAX™ instrument with Check-Direct CPE real-time PCR for the detection of carbapenemase genes from rectal swabs, in a setting with endemic dissemination of carbapenemase-producing *Enterobacteriaceae* Antonelli *et al.*, DMID, 2016

Florence, Italy
557 rectal swabs

Limit of detection (LOD)

Target	LOD BDMix (CFU/ml)	LOD CPMix (CFU/ml)
bla_{KPC}	9×10^{5}	9×10^{3}
bla_{VIM}	4.5×10^{2}	4.5×10^{2}
bla_{OXA-48}	8.5×10^{1}	8.5×10^{1}
bla_{NDM}	7.3×10^{4}	7.3×10^{1}

Culture *vs.* C-D direct CPE (2 ≠ master mix)

Summary of the results

ChromID CARBA SMART (w/wo broth enrichment) → [5 samples not detected]

		Colonization status	
		Positive (n. 29)	*Negative (n. 528)*
CHSM and BERM	*Positive*	24 (82.8 %)[a]	0
	Negative	5 (17.2 %)	528 (100 %)
BDMix	*Positive*	27 (93.1 %)	14 (2.7 %)
18 (3%) unresolved	*Negative*	2 (6.9 %)	496 (93.9 %)
	Unresolved	-	18 (3.4 %)
CPMix	*Positive*	29 (100 %)	15 (2.9 %)
6 (1%) unresolved	*Negative*	0	507 (96.0 %)
	Unresolved	-	6 (1.1 %)

TAT from 18-24 hrs (direct culture) or 48 hrs (broth enrichment) to 3 hrs

23 KPC
5 VIM
1 OXA-48

Performance of Check-Direct CPE screening for BD MAX

	Positives for bla_{KPC}		Positives for bla_{VIM}		Positives for bla_{OXA-48}		Positives for bla_{NDM}	
	BDMix	CPMix	BDMix	CPMix	BDMix	CPMix	BDMix	CPMix
sensitivity	91,3%	100%	100%	100%	100%	100%	-	-
specificity	98,6%	99,6%	99,3%	97,6%	99,3%	99,6%	100%	100%
PLR	65.21	250	142.86	41.67	-[a]	-	-	-
NLR	0.09	0	0	0	-	-	-	-

Cepheid GeneXpert

- Add aliquot to elution, vortex, transfer to port S
- Insert cartridge to station (overall, **1 min**)
- Run time (**<1 h**)

Real-time multiplex PCR

- Smart fluidic system
- Filtering and Sonication (DNA)
- Fluorescent-labeled hybr. probes (6 colors)
- Internal control

Xpert® Carba-R

KPC
NDM
OXA-48-like
VIM
IMP-1

Xpert® vanA

Xpert® MRSA/SA BC

~50 Euro

MSSA
MRSA
CoNS

spa, mecA
SCCmec-orfX

Multisite Evaluation of Cepheid Xpert Carba-R Assay for Detection of Carbapenemase-Producing Organisms in Rectal Swabs

JCM, 54:7; 2016

M. Tato,[a] P. Ruiz-Garbajosa,[a] M. Traczewski,[b] A. Dodgson,[c] A. McEwan,[c] R. Humphries,[d] J. Hindler,[d] J. Veltman,[e] H. Wang,[f] R. Cantón[a]

4 centers (2 USA, 1 UK, 1 Spain)
July 2013 – Feb 2014
633 samples

50% of them spiked at 1.1 x 10^2 to 1.2 x 10^3 CFU/swab (LOD of Xpert)

Results (obtained in 32-48 min)

Xpert Carba-R assay result	Clinical specimens (n = 383)	Contrived specimens (n = 250)	All specimens (n = 633)
Positive (single and/or combined targets)	42	107	149 (23.5%)
IMP-1	0	25	25
VIM	2	24	26
NDM	2	23	25
KPC	13	19	32
OXA-48	20	15	35
VIM + OXA-48	4	0	4
NDM + KPC	1	0	1
IMP-1 + NDM	0	1	1
Negative	341	143	484

Results by individual targets

Xpert Carba-R assay	Reference method (culture plus sequencing)						
	IMP-1	VIM	NDM	KPC	OXA-48	Negative	Total
IMP-1	26	0	0	0	0	0	26
VIM	0	29	0	0	0	1	30
NDM	0	0	26	0	0	1	27
KPC	0	0	0	29	0	4	33
OXA-48	0	0	0	0	38	1	39
Negative	1	2	0	1	2	3,004	3,010
Total	27	31	26	30	40	3,011	3,165

6 out of 154 specimens not detected by Xpert
6 cases of multiple genes (4 not detected by ref. method)

Performance *vs.* reference method

Xpert Carba-R assay	Reference method (culture plus sequencing)		
	No. positive	No. negative	Total No.
Positive	142	7	149
Negative	6	478	484
Total	148	485	633

Performance for different targets

Target gene	Sensitivity (% [95% CI])	Specificity (% [95% CI])	PPV (%)	NPV (%)
IMP-1	96.3 (81.0–99.9)	100 (99.4–100)	100	99.8
VIM	93.5 (78.6–99.2)	99.8 (99.1–100)	96.7	99.7
NDM	100 (86.8–100)	99.8 (99.1–100)	96.3	100
KPC	96.7 (82.8–99.9)	99.3 (98.3–99.8)	87.9	99.8
OXA-48	95.0 (83.1–99.4)	99.8 (99.1–100)	97.4	99.7

Sensitivity: 96.6%
Specificity: 98.6%

Comparison of the Next-Generation Xpert MRSA/SA BC Assay and the GeneOhm StaphSR Assay to Routine Culture for Identification of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* in Positive-Blood-Culture Broths — Buchan BW *et al.*, J Clin Microb, 53:3; 2015

Multicenter study (8 hospitals, USA)
795 BCs (30%, *S. aureus*; 13% MRSA)

Initial test validity
- Xpert: 96.1%
- GeneOhm: 96.3%

Retest:
- Xpert: 99.6%
- GeneOhm: 98.1%

Identification of *Staphylococcus aureus*

Test	Site	Total no. of specimens tested	No. with result[a]: TP	FP	TN	FN	Sensitivity (% [95% CI])[b]	Specificity (% [95% CI])
Xpert MRSA/SA BC	A	63	19	0	44	0	100 (82.4–100)	100 (92.0–100)
	B	91	32	0	59	0	100 (89.1–100)	100 (93.9–100)
	C	44	17	0	27	0	100 (80.5–100)	100 (87.2–100)
	D	70	26	0	44	0	100 (86.8–100)	100 (92.0–100)
	E	130	27	2[c]	100	1[d]	96.4 (79.7–99.8)	98.0 (92.4–99.6)
	F	211	65	1[e]	145	0	100 (94.5–100)	99.3 (96.2–100)
	G	126	31	0	95	0	100 (88.8–100)	100 (96.2–100)
	H	57	18	0	39	0	100 (81.5–100)	100 (91.0–100)
	Total	792	235	3	553	1	99.6 (97.7–99.9)	99.5 (98.4–99.9)
GeneOhm StaphSR	Total	782	234	19	527	2	99.2 (97.0–99.9)	96.5 (94.6–97.9)

Statistically equivalent

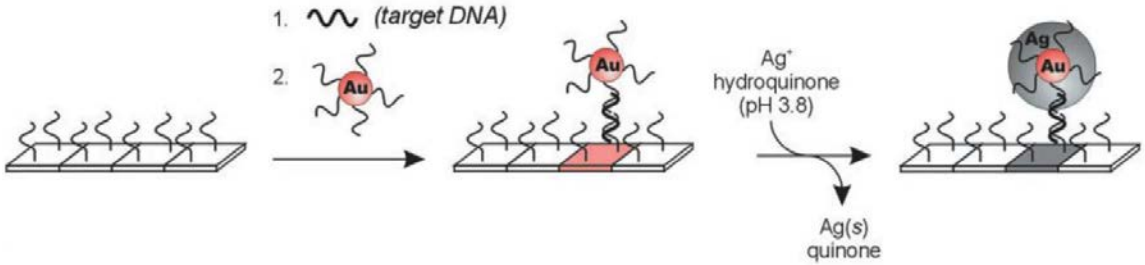
Identification of MRSA

Test	Site	Total no. of specimens tested	No. with result[a]: TP	FP	TN	FN	Sensitivity (% [95% CI])[b]	Specificity (% [95% CI])
Xpert MRSA/SA BC	A	63	11	0	52	0	100 (71.5–100)	100 (93.2–100)
	B	91	16	0	75	0	100 (79.4–100)	100 (95.2–100)
	C	44	7	0	36	1	87.5 (47.3–99.7)	100 (90.3–100)
	D	70	11	1[c]	58	0	100 (71.5–100)	98.3 (90.9–100)
	E	130	10	1[d]	119	0	100 (69.2–100)	99.2 (95.4–100)
	F	211	25	1[c]	185	0	100 (86.3–100)	99.5 (97.0–100)
	G	126	19	0	106	1	95.0 (73.0–99.7)	100 (96.2–100)
	H	57	4	0	53	0	100 (81.5–100)	100 (91.6–100)
	Total	792	103	3	684	2[e]	98.1 (93.3–99.8)	99.6 (98.7–99.9)
GeneOhm StaphSR	Total	782	99	15	662	6[i]	94.3 (88.0–97.9)	97.8 (96.4–98.8)

Statistically equivalent

Verigene, Nanosphere

- Load cartridge, consumables, and sample (**5 min**)
- Automated sample preparation and processing
- Place slide from cartridge in reader (**2.5 hrs**)



Microarray approach by using Au-nanoprobe as reporter and silver reduction to enhance signal

Gram-negatives cartridge

Species	Genus	Resistance
Escherichia coli	*Acinetobacter* spp.	CTX-M (ESBL)
Klebsiella pneumoniae	*Citrobacter* spp.	IMP (carbapenemase)
Klebsiella oxytoca	*Enterobacter* spp.	KPC (carbapenemase)
Pseudomonas aeruginosa	*Proteus* spp.	NDM (carbapenemase)
Serratia marcescens		OXA (carbapenemase)
		VIM (carbapenemase)

Gram-positives cartridge

Species	Genus	Resistance
Staphylococcus aureus	*Staphylococcus* spp.	*mecA* (methicillin)
Staphylococcus epidermidis	*Streptococcus* spp.	*vanA* (vancomycin)
Staphylococcus lugdunensis	*Micrococcus* spp.	*vanB* (vancomycin)
Streptococcus anginosus Group	*Listeria* spp.	
Streptococcus agalactiae		
Streptococcus pneumoniae		
Streptococcus pyogenes		
Enterococcus faecalis		
Enterococcus faecium		

~50 Euro

Performance Evaluation of the Verigene Gram-Positive and Gram-Negative Blood Culture Test for Direct Identification of Bacteria and Their Resistance Determinants from Positive Blood Cultures in Hong Kong

Gilman K.H. Siu *et al.*, Plos One, Oct 2015

Multicenter study (4 hospitals)

364 BCs (114 Gram-pos; 250 Gram-neg)

Jan 2014 – May 2014

Initial test validity: 95.6%

ID for Gram-positives (agreement 89.6%)

Organisms	No. (%) of isolates Total	Correctly identified	Not detected	Misidentified	No Call	Sensitivity (%)	Specificity (%)
Staphylococcus spp.	73 (60.3)	69 (94.5)	-	-	4	100	100
S. aureus	48 (39.7)	47 (97.9)	-	-	1	100	100
S. epidermidis	4 (3.3)	4 (100)	-	-	-	100	100
Streptococcus spp.	27 (22.3)	25 (92.6)	-	-	2	100	98.9
S. pyogenes	2 (1.7)	2 (100)	-	-	-	100	100
S. agalactiae	3 (2.5)	3 (100)	-	-	-	100	100
S. anginosus gr.	1 (0.8)	1 (100)	-	-	-	100	100
S. pneumoniae	7 (5.8)	7 (100)	-	-	-	100	99.1
E. faecalis	7 (5.8)	4 (57.1)	2 (28.6)	-	1	66.7	100
E. faecium	5 (4.1)	2 (40)	2 (40)	-	1	50	100
Listeria	1 (0.8)	1 (100)	-	-	-	100	100

ID for Gram-negatives (agreement 90.5%)

Organisms	No. (%) of isolates Total	Correctly identified	Not detected	Misidentified	No Call	Sensitivity (%)	Specificity (%)
E.coli	165 (59.1)	158 (95.8)	6 (3.6)	1[c]	-	95.8	100
K. pneumoniae	52 (18.6)	36 (69.2)	14 (27)[d]	2[e]	-	69.2[d]	100
					K. variicola 80%		
P. aeruginosa	17 (6.1)	13 (76.5)	3 (17.6)	-	1	81.3	100
Proteus spp.	10 (3.6)	9 (90)	-	-	1	100	100
Enterobacter spp.	10 (3.6)	8 (80)	-	-	2	100	99.3
Acinetobacter spp.	4 (1.4)	4 (100)	-	-	-	100	100
K. oxytoca	3 (1.1)	-	3 (100)	-	-	0	99.3
Citrobacter spp.	1 (0.4)	1 (100)	-	-	-	100	100
Serratia marcescens	1 (0.4)	1 (100)	-	-	-	100	100

A. hydrophila, M. morganii, Salmonella spp., A. faecalis, B. pseudomallei, H. influenzae, R. planticola, P. putida, S. maltophilia **not detectable with the system** (n=16; 5.5%)

Drug-Resistant Organisms

Drug resistant Organisms	No. of isolates Total	Correctly Detected	Not Detected	No Call	Sensitivity	Specificity (%)
Gram Positive						
MRSA	27	26	0	1	100	100
MRSE	4	4	0	0	100	100
VRE	1	1	0	0	100	100
Gram Negative						
Cefotaxime resistant *Enterobacteriaceae* (including ESBL producers)	61	38	22	1	63.3	100
ESBL producing *Enterobacteriaceae*	46	38	7	1	84.4	100
MDR *Acinetobacter*	3	3	0	0	100	100
Carbapenem-resistant *Psuedomonas*	2	0	2	0	0	100
Total	**98**	**72**	**24**	**2**	**75.0**	**100**

Time to Results (40-99 hrs faster than routine)

Organisms	No. of Isolates[b]	Average Time to identification by Culture-Based Method (h)	Average Time to Result by Verigene Test (h)	Δ Time to Result[a] Average (h)	Range (h)	*p*-value
Staphylococcus spp.	23	63.76	2.35	61.41	22.65–494.65	*p*<0.001
MSSA	7	49	2.35	46.65	22.65–79.65	*p* = 0.012
MRSA	10	93.45	2.35	91.1	23.65–494.65	*p*<0.001
CNS	8	57.19	2.35	54.84	29.65–97.65	*p*<0.001
Streptococcus spp.	13	63.86	2.35	61.51	22.65–128.15	*p*<0.001
S. pneumoniae	3	42.83	2.35	40.48	22.65–56.65	p = 0.017
β-haemolytic Strept.	4	71.62	2.35	69.27	33.65–128.15	*p*<0.001
Viridians Group	6	55.08	2.35	52.73	25.65–83.65	*p*<0.001
Enterococcus spp.	2	78.5	2.35	76.15	53.15–99.15	*p*<0.001
VSE	1	55.5	2.35	53.15	n/a	n/a
VRE	1	101.5	2.35	99.15	n/a	n/a
Enterobacteriaceae	79	45.98	1.88	44.1	18.12–104.12	*P*<0.001
P. aeruginosa	7	52.51	1.88	50.63	28.62–94.62	*p*<0.001
Acinetobacter spp.	1	76	1.88	74.12	n/a	n/a

Clinical Impact of Laboratory Implementation of Verigene BC-GN Microarray-Based Assay for Detection of Gram-Negative Bacteria in Positive Blood Cultures

Walker T. *et al.*, J Clin Microbiol; 54:7, 2016

Los Angeles
May 2013 - Nov 2013
Dec 2014 – May 2015

6-month period before Verigene (n=98) *vs.* 6-month period after Verigene (n=97)

Clinical outcome

	Pre-BC-GN	Post-BC-GN	*P* value
Mean time from initial Gram stain to BC-GN identification, h	NA[a]	3.5	NA
Mean time from initial Gram stain to organism identification, h	37.9	10.9	<0.001[b]
Mean time from initial Gram stain to effective therapy, h			
All cases	10.2	6.5	0.12[b]
Cases on suboptimal empirical therapy	30.3	19.1	0.12[b]
No. of cases in which therapy was de-escalated	33	37	0.66[c]
Mean time from initial Gram stain to de-escalation, h	40.9	34.1	0.14[b]
Recurrence of bacteremia, no. (%)	8 (8.2)	3 (3.1)	0.21[c]
Mean total length of stay in hospital, days	15.2	18.0	0.52[b]
Mean length of hospital stay after positive culture, days	9.7	9.4	0.87[b]
Mean length of stay in ICU, days	16.2	12.0	0.03[b]
30-day mortality, no. (%)	19 (19.2)	8 (8.1)	0.04[c]
ESBL cases, no.	15	11	0.53[c]
Length of stay in hospital, days	12.0	13.5	0.59[b]
Mean time to effective therapy, h (no.)[d]	41.4 (9)	7.3 (9)	0.04[b]
30-day mortality, no. (%)	4 (26.7)	0 (0)	0.11[c]

Saved 11,661 USD/case ←

Factors associated to length of ICU stay

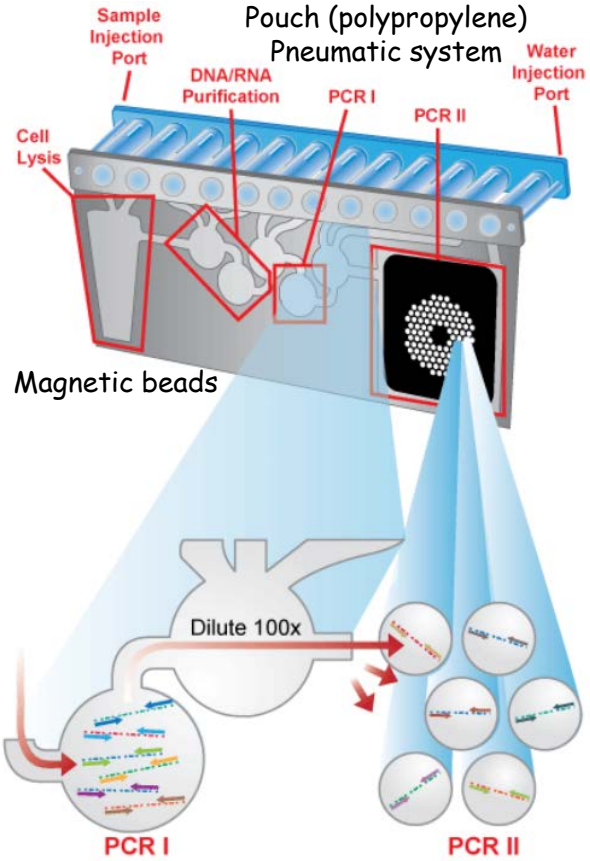
	Univariate analysis[b]			Multivariate analysis[b]		
Factor	HR	95% CI	*P* value	HR	95% CI	*P* value
Intervention	1.62	1.03–2.54	0.04	1.79	1.07–2.98	0.03
Preinfection length of stay	0.97	0.95–0.99	0.001	0.97	0.95–0.98	0.0001
Age	1.01	0.99–1.03	0.22	1.0	0.98–1.01	0.90
Sex (female)	0.64	0.40–1.03	0.06	0.69	0.41–1.15	0.15
Time to effective therapy	1.01	0.78–1.32	0.94	1.02	0.75–1.38	0.90
Disease severity (APACHE II)	1.00	0.97–1.02	0.74	0.97	0.93–1.0	0.06
Mortality	1.03	0.57–1.65	0.91	1.43	0.83–2.48	0.20

Multiv. log. regression for association with 30-d mortality

Factor	Odds ratio	95% CI	*P* value
Intervention	0.81	0.67–0.98	0.03
Length of stay, ICU	1.00	1.00–1.00	0.94
Age	1.01	1.00–1.01	0.11
Sex (female)	1.07	0.88–1.30	0.48
APACHE II	1.01	0.99–1.02	0.36

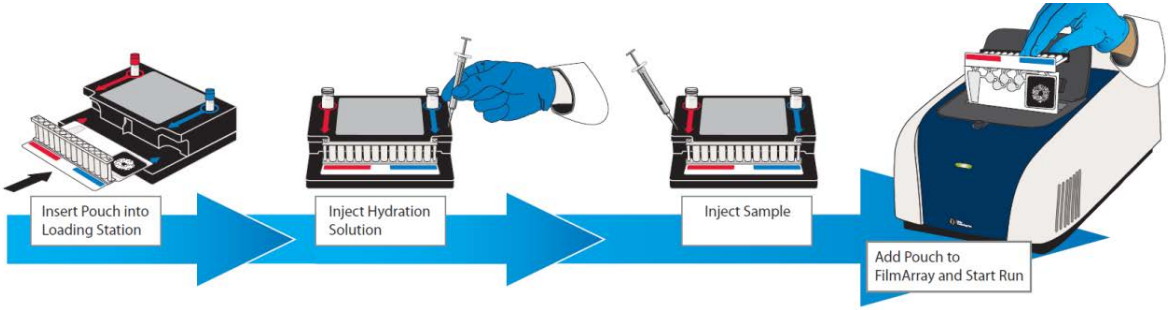
BioFire FilmArray

- Preparation of the pouch
- Add pouch to FilmArray station (overall, **2 min**)
- Run time of about **1 h**



Multiplex PCR
(RT-PCR for RNA target)

Multiplex real-time PCR
and Melting analysis



FilmArray® BCID Panel targets:

~100 Euro

Gram+ Bacteria	Gram– Bacteria
Enterococcus *Listeria monocytogenes* *Staphylococcus* *Staphylococcus aureus* *Streptococcus* *Streptococcus agalactiae* *Streptococcus pyogenes* *Streptococcus pneumoniae*	*Acinetobacter baumanniiHaemophilus influenzae* *Neisseria meningitidis* *Pseudomonas aeruginosa* *Enterobacteriaceae* *Enterobacter cloacae complex* *Escherichia coli* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Proteus* *Serratia marcescens*
Yeast	**Antibiotic Resistance**
Candida albicans *Candida glabrata* *Candida krusei* *Candida parapsilosis* *Candida tropicalis*	mecA - methicillin resistance vanA/B - vancomycin resistance KPC - carbapenem resistance

Species ID

	Isolates detected[a]: BCID/comparator		No. of results: BCID/comparator					
	Clinical arm	Seeded arm	TP +/+	FP +/−	FN −/+	TN −/−	Sensitivity or PPA[b]: TP/(TP + FN) (%)	Specificity or NPA[b]: TN/(TN + FP) (%)
Gram-positive bacteria								
Enterococcus	102/101	29/29	127	4	3	2,073	127/130 (97.7)	2,073/2,077 (99.8)
L. monocytogenes	0/0	36/36	36	0	0	2,171	36/36 (100)	2,171/2,171 (100)
Staphylococcus	780/797	2/1	770	12	28	1,397	770/798 (96.5)	1,397/1,409 (99.1)
S. aureus	257/257	0/0	253	4	4	1,946	253/257 (98.4)	1,946/1,950 (99.8)
Streptococcus	140/141	63/62	198	5	5	1,999	198/203 (97.5)	1,999/2,004 (99.8)
S. agalactiae (group B)	18/18	18/18	36	0	0	2,171	36/36 (100)	2,171/2,171 (100)
S. pneumoniae	26/25	12/12	36	2	1	2,168	36/37 (97.3)	2,168/2,170 (99.9)
S. pyogenes (group A)	8/7	31/31	38	1	0	2,168	38/38 (100)	2,168/2,169 (99.9)
Total	1,331/1,346	191/189	1,494	28	41	16,093	1,494/1,535 (97.3)	16,093/16,121 (99.8)
Gram-negative bacteria								
A. baumannii	16/14	40/37	51	5	0	2,151	51/51 (100)	2,151/2,156 (99.8)
Enterobacteriaceae	307/310	187/188	490	4	8	1,705	490/498 (98.4)	1,705/1,709 (99.8)
E. cloacae complex	24/22	17/17	38	3	1	2,165	38/39 (97.4)	2,165/2,168 (99.9)
E. coli	149/148	6/5	150	5	3	2,049	150/153 (98.0)	2,049/2,054 (99.8)
K. oxytoca	6/6	54/58	59	1	5	2,142	59/64 (92.2)[c] 98%	2,142/2,143 (99.9)
K. pneumoniae	74/71	37/34	102	9	3	2,093	102/105 (97.1)	2,093/2,102 (99.6)
Proteus	22/22	17/17	39	0	0	2,168	39/39 (100)	2,168/2,168 (100)
S. marcescens	22/22	55/55	76	1	1	2,129	76/77 (98.7)	2,129/2,130 (99.9)
H. influenzae	8/8	35/35	43	0	0	2,164	43/43 (100)	2,164/2,164 (100)
N. meningitidis	1/1	35/35	36	0	0	2,171	36/36 (100)	2,171/2,171 (100)
P. aeruginosa	52/52	0/0	51	1	1	2,154	51/52 (98.1)	2,154/2,155 (99.9)
Total	681/676	483/481	1135	29	22	23,091	1,135/1,157 (98.1)	23,091/23,120 (99.9)

R. ornithinolytica

Only 1.9% needed repeat testing

Evaluation of the FilmArray Blood Culture Identification Panel: Results of a Multicenter Controlled Trial

Salimnia H.. *et al.*, J Clin Microbiol; 54:3, 2016

8 centers in USA

July 2012 - Feb 2014

2,207 BC samples

- 1,568 clinical
- 639 seeded

FilmArray performed:

- BC+, within 8 hrs
  - 52% clinical samples
  - 66% seeded cultures
- Aliquot frozen (<8 hrs)

Drug-Resistant Organisms

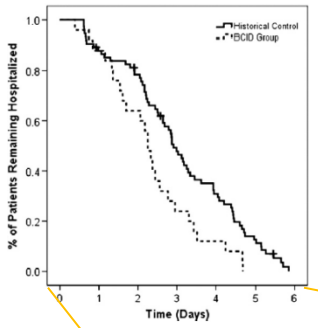
gene(s)	Isolates detected: BCID/comparator Clinical arm	Seeded arm	No. of results: BCID/comparator TP +/+	FP +/−	FN −/+	TN −/−	Sensitivity or PPA[a]: TP/(TP + FN) (%)	Specificity or NPA[a]: TN/(TN + FN) (%)
mecA in association with:								
All *Staphylococcus* isolates detected[b]	491/494	2/2	488	5	8	281	488/496 (98.4)	281/286 (98.3)
Staphylococcus and *S. aureus* isolates detected	137/139	0/0	137	0	2	118	137/139 (98.6)	118/118 (100)
vanA/B in association with *Enterococcus* isolates detected	36/36	28/28	64	0	0	67	64/64 (100)[c]	67/67 (100)
bla_{KPC} in association with *Enterobacteriaceae* and/or *A. baumannii* and/or *P. aeruginosa* isolates detected	6/6[d]	33/33	39	0	0	558	39/39 (100)[e]	558/558 (100)

Clinical and economic impact of antimicrobial stewardship interventions with the FilmArray blood culture identification panel *Pardo J et al.*, DMID; 84, 2016

Gainesville, Florida

Intervention group (FilmArray)
(Aug 2013 – Jan 2014; n=84)
vs.
Historical control group
(Jan 2012 – Jun 2013; n=252)

55% CoNS



Time to discharge after CoNS-contaminated BCs

	All Patients ($N = 336$)			Discharged Within 6 Days of CoNS Contamination ($N = 100$)		
Variable	Control group ($N = 252$)	BCID group ($N = 84$)	P	Control group ($N = 75$)	BCID group ($N = 25$)	P
Median LOS (days [IQR])	7.9 (3.8–16.3)	7.4 (3–11.7)	0.178	2.9 (2.1–4.4)	2.3 (1.5–3.1)	0.008
Median ICU Days (IQR)	0 (0–5)	0 (0–1)	0.026	0 (0–0)	0 (0–0)	0.838
In-hospital mortality (No. [%])	37 (15)	5 (6)	0.036	4 (6)	0 (0)	0.239
Median Costs ($ [IQR])						
Total	15324 (5517–37305)	12241 (3080–21027)	0.030	3370 (1537–5190)	1645 (585–2952)	0.016
Pharmacy	2119 (598–5503)	1538 (366–3824)	0.073	429 (157–712)	214 (75–361)	0.010
Ward room	2806 (0–7427)	3972 (947–8041)	0.514	1852 (891–2778)	947 (0–1828)	0.025
ICU room	1222 (0–10218)	0 (0–3756)	0.008	0 (0–0)	0 (0–0)	0.659
Laboratory	708 (241–1804)	656 (254–1445)	0.692	160 (83–253)	204 (160–321)	0.020

129 USD

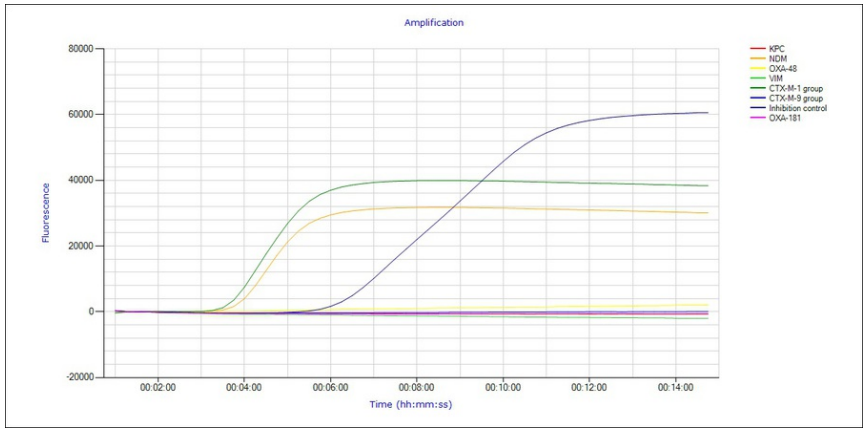
Unnecessary vancomycin use

	MSSA (N = 32)			CoNS Contaminants (N = 184)		
Variable	Control Group (N = 24)	BCID Group (N = 8)	P	Control Group (N = 138)	BCID Group (N = 46)	P
Vancomycin started (No. [%])	24 (100)	8 (100)	>0.99	100 (72)	32 (70)	0.705
1 dose only (No. [%])	0/24 (0)	4/8 (50)	0.002	12/100 (12)	7/32 (22)	0.133
Median duration (h [IQR])	66.0 (54.1–78.1)	14.0 (1–20)	<0.001	66.9 (37.6–135)	39.3 (14.7 –96.5)	0.100

Amplex Eazyplex

Genie II platform

- Preparation (**5 min**)
- No DNA extraction
- Run time (**<30 min**)



LAMP
Loop-mediated isothermal Amplification

Real-time fluorescent measurement

~55 Euro

eazyplex® MRSA

S. aureus
mecA
mecC
S. epidermidis

eazyplex® VRE

eazyplex® SuperBug mcr-1

eazyplex® SuperBug CRE

KPC,
NDM,
OXA-48
and OXA 181,
VIM, as well as
CTX-M-1 and
CTX-M-9 group

eazyplex® SuperBug complete

Version A	Version B
KPC	KPC
NDM	NDM
OXA-48	OXA-48
VIM	VIM
OXA-23 group	OXA-23 group
OXA-40 group	OXA-40 group
OXA-58 group	OXA-181

Extended-spectrum β-lactamase (ESBL) detection directly from urine samples with the rapid isothermal amplification-based eazyplex® SuperBug CRE assay: Proof of concept☆

Hinic V. *et al.*, J Microb Meth; 119, 2015

No	Species (phenotype) isolated from urine	Quantity in urine culture (CFU/ml)	eazyplex® SuperBug CRE: Bacterial isolate – Target detected	Bacterial isolate – Threshold time (min:sec)	Urine – Target detected	Urine – Threshold time (min:sec)
1	*Escherichia coli* (ESBL)	10^6	CTX-M-1 group	04:30	CTX-M-1 group	05:45
2	*Escherichia coli* (ESBL)	10^6	CTX-M-1 group	04:45	CTX-M-1 group	05:30
3	*Escherichia coli* (ESBL)	10^5	CTX-M-1 group	04:30	CTX-M-1 group	08:45
4	*Escherichia coli* (ESBL)	10^4	CTX-M-1 group	04:45	CTX-M-1 group	09:00
5	*Escherichia coli* (ESBL), *Enterococcus faecalis*	10^6, 10^5	CTX-M-1 group	05:00	CTX-M-1 group	05:15
6	*Escherichia coli* (ESBL)	10^4	CTX-M-1 group	04:30	CTX-M-1 group	10:45
7	*Escherichia coli* (ESBL), *Escherichia coli* (no β-lactamase)	10^4, 10^4	CTX-M-1 group	04:54	CTX-M-1 group	08:45
8	*Escherichia coli* (ESBL)	10^6	CTX-M-1 group	05:15	CTX-M-1 group	07:30
9	*Escherichia coli* (ESBL)	10^6	CTX-M-1 group	04:45	CTX-M-1 group	07:00
10	*Escherichia coli* (ESBL), *Escherichia coli* (no β-lactamase)	10^6, 10^5	CTX-M-1 group	04:30	CTX-M-1 group	06:45
11	*Escherichia coli* (ESBL), *Enterococcus faecium*	10^4, 10^5	CTX-M-1 group	04:45	CTX-M-1 group	11:00
12	*Escherichia coli* (ESBL)	10^5	CTX-M-1 group	04:30	CTX-M-1 group	07:30
13	*Escherichia coli* (ESBL), gram-positive flora	10^5, 10^4	CTX-M-1 group	04:30	CTX-M-1 group	07:45
14	*Escherichia coli* (ESBL)	10^6	CTX-M-1 group	04:15	CTX-M-1 group	05:30
15	*Escherichia coli* (ESBL)	10^5	CTX-M-1 group	05:15	CTX-M-1 group	06:00
16	*Escherichia coli* (ESBL)	10^6	CTX-M-1 group	05:00	CTX-M-1 group	05:15
17	*Escherichia coli* (ESBL), *Klebsiella pneumoniae* (no β-lactamase)	10^4, 10^3	CTX-M-1-group	05:15	CTX-M-1 group	06:00
18	*Escherichia coli* (ESBL), *Enterococcus faecalis*	10^4, 10^4	CTX-M-1-group	05:30	CTX-M-1 group	14:00
19	*Escherichia coli* (ESBL)	10^6	CTX-M-1-group	04:15	CTX-M-1 group	04:30
20	*Klebsiella pneumoniae* (ESBL)	10^6	CTX-M-1-group	04:30	CTX-M-1 group	04:30
21	*Klebsiella pneumoniae* (ESBL)	10^6	CTX-M-1 group	05:00	CTX-M-1 group	06:30
22	*Klebsiella pneumoniae* (ESBL)	10^4	CTX-M-1 group	04:45	CTX-M-1 group	08:30
23	*Escherichia coli* (ESBL)	10^6	CTX-M-9 group	05:45	CTX-M-9 group	06:30
24	*Escherichia coli* (ESBL)	10^5	CTX-M-9 group	06:00	CTX-M-9 group	12:45
25	*Escherichia coli* (ESBL), gram-positive flora	10^6, 10^5	CTX-M-9 group	05:15	CTX-M-9 group	05:45
26	*Escherichia coli* (ESBL)	10^5	CTX-M-9 group	04:45	CTX-M-9 group	08:15
27	*Escherichia coli* (ESBL)	10^4	CTX-M-9 group	05:30	CTX-M-9 group	12:30
28	*Escherichia coli* (ESBL)	10^6	CTX-M-9 group	07:30	CTX-M-9 group	11:15
29	*Escherichia coli* (ESBL), mixed flora 2×	10^5, 10^3	CTX-M-9 group	08:15	CTX-M-9 group	13:45
30	*Klebsiella pneumoniae* (ESBL)	10^6	CTX-M-9-group	06:15	CTX-M-9 group	07:30
31	*Escherichia coli* (AmpC)	10^5	negative		negative	
32	*Escherichia coli* (AmpC), gram-positive flora 2×	10^5, 10^4	negative		negative	
33	*Escherichia coli* (AmpC)	10^6	negative		negative	
34	*Escherichia coli* (AmpC), gram-positive flora	10^4, 10^5	negative		negative	
35	*Enterobacter cloacae* (AmpC)	10^5	negative		negative	
36	Gram-positive flora 4×	10^5			negative	
37	*Enterococcus faecalis*	10^5			negative	
38	*Citrobacter koseri* (no β-lactamase), *Aerococcus urinae*	10^6, 10^4			negative	
39	*Lactobacillus crispatus*, gram-positive flora 3×	10^5, 10^4			negative	
40	*Candida glabrata*	10^5			negative	
41	*Escherichia coli* (no β-lactamase)	10^5			KPC, NDM, VIM, OXA-48, OXA-181, CTX-M-1 group, CTX-M-9 group[a]	16:30, 17:00, 16:45, 18:30, 19:15, 16:00, 19:15
42	*Pseudomonas aeruginosa*, *Morganella morganii* (AmpC)	10^5, 10^6			negative	
43	*Klebsiella pneumoniae* (no β-lactamase)	10^6			negative	
44	*Proteus mirabilis* (no β-lactamase)	10^5			negative	
45	*Enterococcus faecalis*, *Citrobacter freundii*-group (AmpC), *Pseudomonas aeruginosa*, *Achromobacter xylosoxidans*	10^5, 10^5, 10^4, 10^5			negative	
46	*Escherichia coli* (no β-lactamase)	10^6			negative	
47	*Escherichia coli* (no β-lactamase)	10^6			KPC, NDM, VIM, OXA-48, OXA-181,	08:15, 08:30, 08:30, 08:30, 09:30, 08:00,

Avg. 8 min

50 urine samples

33 ESBL+ and/or carbapenemases
- Vitek 2 and Etest ESBL/AmpC strips
- Eazyplex on colonies

LOD in Urine

Determination of analytical sensitivity of direct urine testing by using a CTX-M-1-group-producing *E. coli* strain (701541/14).

No. of CFU/ml	Threshold time (min:sec)	Result
10^5	08:45	positive
10^4	11:00	positive
10^3	16:00	positive
10^2	0	negative

First run with 50 urines:

- 2 invalid results
- 1 false-positive NDM (19 min)

Sensitivity: 100%
Specificity: 97.9%

Evaluation of loop-mediated isothermal amplification for the rapid identification of bacteria and resistance determinants in positive blood cultures

Rödel J. *et al.*, Eur J Clin Microbiol Infect Dis; Jan 2017

Jena, Germany
April to Aug 2015
370 BCs + (of which 140 G+)

eazyplex® MRSA

Table 1 Analytes detected by the eazyplex® assays used in this study[a]

MRSA	GP	GN
S. aureus	*E. faecalis*	*E. coli*
S. epidermidis	*Enterococcus* spp.	*K. pneumoniae*
mecA	*S. pneumoniae*	Enterobacteriaceae
mecC	*Streptococcus* spp.	*Pseudomonas* spp.
	vanA	$bla_{\text{CTX-M-1}}$ group
	vanB	$bla_{\text{CTX-M-9}}$ group

In-house LAMP assays (not available)

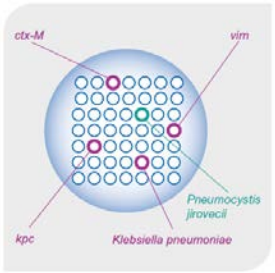
ID § Resistance	True-positive (*n*)	True-negative (*n*)	False-positive (*n*)	False-negative (*n*)	No. positive/no. tested (%) (95% CI[a])	
					Sensitivity	Specificity
MRSA						
S. aureus	31	106	2	0	100 (88.8–100)	98.2 (93.5–99.8)
MRSA[d]	6	25	0	0	100 (54.1–100)	100 (86.3–100)
S. epidermidis	72	60	1	6	92.3 (84–97.1)	98.4 (91.2–100)
mecA (CoNS)	73	30	0	3	96 (88.9–99.2)	100 (88.4–100)
GP						
E. faecalis	12	58	1	0	100 (73.5–100)	98.3 (90.9–100)
Enterococcus spp.	41	27	2	1	97.6 (87.4–99.9)	93.1 (77.2–99.1)
VRE[e]	3	39	0	0	100 (29.2–100)	100 (91–100)
S. pneumoniae	2	69	0	0	100 (15.8–100)	100 (94.8–100)
Streptococcus spp.	20	50	0	1	95.2 (76.2–99.9)	100 (92.9–100)
GN						
E. coli	97	58	2	0	100 (96.3–100)	96.7 (88.5–99.6)
E. coli CTX-M[f]	17	80	0	0	100 (89.5–100)	100 (95.5–100)
E. coli ESBL[g]	17	78	0	2	89.5 (66.9–98.7)	100 (95.3–100)
K. pneumoniae	11	143	2	1	91.7 (61.5–99.8)	98.6 (95.1–99.8)
K. pneumoniae ESBL[h]	4	7	0	0	100 (39.8–100)	100 (59–100)
Enterobacteriaceae	134	12	11	2	98.5 (94.8–99.8)	52.2 (30.6–73.2)
Pseudomonas spp.	9	148	0	0	100 (66.4–100)	100 (97.5–100)

Time to results	Threshold time [min; mean values (SD)]	
	True-positive	False-positive
MRSA		
S. aureus	7 (1.5)	17
mecA (*S. aureus*)	11.5 (4)	–
S. epidermidis	11.75 (2.75)	18.75
mecA (CoNS)	9.75 (2.5)	–
IC	7.5 (1)	–
GP		
E. faecalis	6.75 (1)	17.5
Enterococcus spp.	10 (4)	17.5
vanA	7 (1)	–
vanB	–	–
S. pneumoniae	7	–
Streptococcus spp.	13.5 (3.25)	–
IC	9.5 (0.75)	–
GN		
E. coli	7.25 (1.75)	15
K. pneumoniae	13.75 (2.5)	10.75
$bla_{\text{CTX-M-1}}$ group[a]	8.5 (4)	–
$bla_{\text{CTX-M-9}}$ group[b]	6.25 (1)	–
Enterobacteriaceae	6.25 (1.75)	15.75 (2.5)
Pseudomonas spp.	8.25 (1.5)	–
IC	9 (1.75)	–

In principle, from +BC ~30 min

Curetis Unyvero

- Sample transferred to Unyvero tube (2 min)
- Lysis (30 min), transfer to cartridge, plus master mix (3 min)
- Overall, time to results (**~5 hrs**)



DNA purification, PCR set up, multiplex PCRs with array detection

Pneumonia panel (P55)

GROUP	PATHOGEN	GENE	RESISTANCE AGAINST
Gram-positive bacteria	*Staphylococcus aureus*	*ctx-M*	3rd generation Cephalosporins
	Streptococcus pneumoniae	*ermB*	Macrolide/Lincosamide
Enterobacteriaceae	*Citrobacter freundii*	*imp*	Carbapenem
	Escherichia coli	*kpc*	Carbapenem
	Enterobacter cloacae complex	*mecA*	Oxacillin
	Enterobacter aerogenes	*mecC*	Oxacillin
	Proteus spp.	*ndm*	Carbapenem
	Klebsiella pneumoniae	*oxa-23*	Carbapenem
	Klebsiella oxytoca	*oxa-24*	Carbapenem
	Klebsiella variicola	*oxa-48*	Carbapenem
	Serratia marcescens	*oxa-58*	Carbapenem
	Morganella morganii	*shv*	Penicillin
Non-fermenting bacteria	*Moraxella catarrhalis*	*sul1*	Sulfonamide
	Pseudomonas aeruginosa	*tem*	Penicillin
	Acinetobacter baumannii compl	*vim*	Carbapenem
	Stenotrophomonas maltophilia	*gyrA83*	Fluoroquinolone
	Legionella pneumophila	*gyrA87*	Fluoroquinolone
Others / Fungi	*Pneumocystis jirovecii*		
	Haemophilus influenzae		
	Mycoplasma pneumoniae		

Blood culture panel (BCU)

GROUP	PATHOGEN	GENE	RESISTANCE AGAINST
Universal Bacteria		*aac(6')aph(2")*	Aminoglycoside
Gram-positive bacteria	*Staphylococcus aureus*	*ermA*	Macrolide/Lincosamide
	Coagulase negative staphylococci	*mecA*	Oxacillin
	Streptococcus spp.	*mecC (LGA251)*	Oxacillin
	Streptococcus agalactiae	*vanA*	Vancomycin
	Streptococcus pneumoniae	*vanB*	Vancomycin
	Streptococcus pyogenes/ dysgalactiae	*aacA4*	Aminoglycoside
	Enterococcus spp.	*ctx-M*	3rd generation Cephalosporine
	Enterococcus faecalis	*kpc*	Carbapenem
	Listeria monocytogenes	*imp*	Carbapenem
Corynebacteriaceae	*Corynebacterium spp.*	*ndm*	Carbapenem
Enterobacteriaceae	*Citrobacter freundii/koseri*	*oxa-23*	Carbapenem
	Escherichia coli	*oxa-24/40*	Carbapenem
	Enterobacter cloacae complex	*oxa-48*	Carbapenem
	Enterobacter aerogenes	*oxa-58*	Carbapenem
	Klebsiella oxytoca	*vim*	Carbapenem
	Klebsiella pneumoniae		
	Klebsiella variicola		
	Proteus spp.		
	Serratia marcescens		
Non-fermenting bacteria	*Acinetobacter baumannii complex*		
	Pseudomonas aeruginosa		
	Stenotrophomonas maltophilia		
other Gram-negative bacteria	*Haemophilus influenzae*		
	Neisseria meningitidis		
Anaerobic bacteria	*Propionibacterium acnes*		

* *Mycobacterium* spp.; Fungi

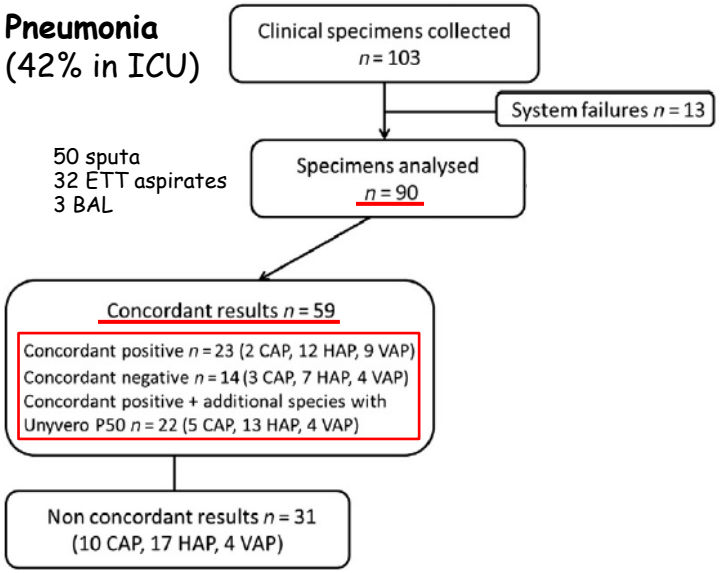
'Sample-in, answer-out'? Evaluation and comprehensive analysis of the Unyvero P50 pneumonia assay

Personne Y. *et al.*, DMID; 86: 2017

London, UK (two hospitals)

Dec 2014 – June 2015

Pneumonia
(42% in ICU)



Sens/Spec: 96% and 33%

Species ID

Target Organism	Routine laboratory	UnyVero P50	True Positive (Routine and Unyvero P50)	False Positive (Unyvero P50 only)	False Negative (Routine only)
A. baumannii	3	10	3	7	0
Enterobacter spp	3	9	3	6	0
E. coli	5	8	5	3	0
H. influenzae	3	7	1	6	2
K. pneumoniae	3	11	3	8	0
M. catarrhalis	3	6	3	3	0
M. morganii	0	1	0	1	0
Proteus spp	1	5	1	4	0
P. aeruginosa	13	19	13	6	0
S. marcescens	6	9	6	3	0
S. aureus	5	11	5	6	0
S. maltophilia	6	27	6	21	0
*S. mitis group**	0	13	0	13	0
L. pneumophila	0	0	0	0	0
C. pneumoniae	0	0	0	0	0
P. jirovecii	0	0	0	0	0
K. oxytoca	0	0	0	0	0
E. faecalis	1	N/A	0	0	1
C. koseri	1	N/A	0	0	1
Negative specimens	42	16	N/A	N/A	N/A

Avg. pathogens 0.59 *vs.* 1.59

Routine
5.5% polymicrobial

Unyvero
49% polymicrobial

It is not a quantitative test

Detection of ARGs

Routine

36% of strains fully susceptible
60% resistant to ≥ 1 antibiotic
40% were MDR

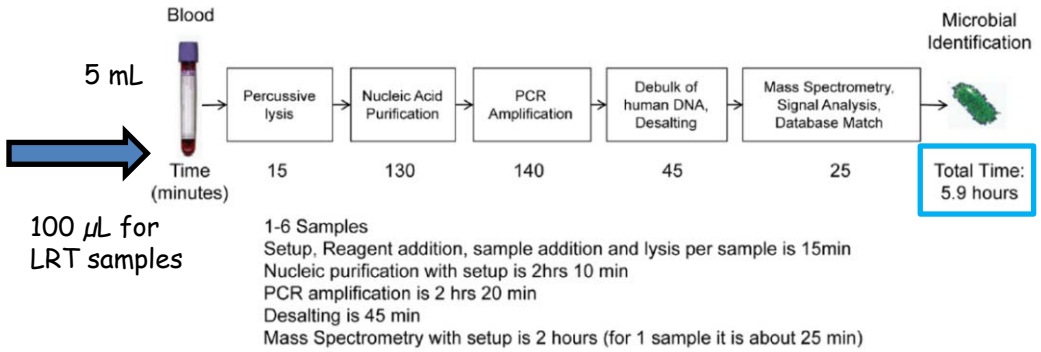
Unyvero

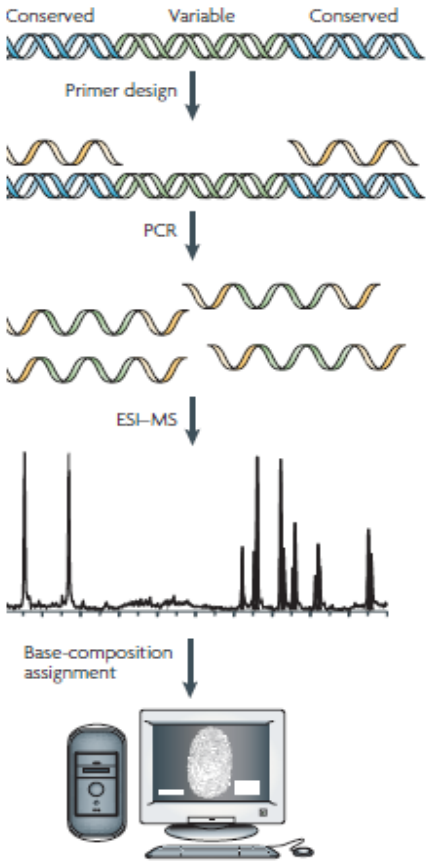
71% of samples with ≥1 ARG
(e.g., bla_{TEM}, *ermB*, *sul1*)

Highly prevalent in commensals

	ESBL producer	MRSA	Fluoroquinolone resistance	Carbapenemase producer	AmpC producer
Routine Microbiology	not detected	n = 1	n = 3 1x *P. aeruginosa* (GyrA 83), 2x *E. coli* (GyrA 83; GyrA 83 + GyrA 87)	n = 4 3 *A. baumannii* (bla_{OXA-23}) 1 *P. aeruginosa* (no enzyme found)	n = 5 3x *S. marcescens* 2x *E. aerogenes* Presumed chromosomal AmpC upregulation
Unyvero P50	n = 1 bla_{CTX-M}	n = 3*	n = 3 1x *P. aeruginosa* (GyrA83, ParC) 2x *E. coli* (GyrA83, GyrA83 + GyrA87)	n = 5 bla_{OXA-51} 2x *A. baumannii* 1x *A. baumanii* + *S. maltophilia* 2x *A. baumannii* + *S. maltophilia* + *S. aureus*	n = 5 2 x bla_{DHA} 1x *M. morganii* + *S. marcescens* 1x *P. aeruginosa* + *S. maltophilia* 3 x bla_{EBC} 2x Enterobacter spp. 1x Enterobacter spp + *M. catarrhalis*
Concordance	No	1/3	2/3	No	No

Abbott IRIDICA PCR-ESI MS system





Ibis T5000, Abbott PLEX-ID

780 Bacteria and Candida, 4 Resistance Markers	Whole blood	IRIDICA BAC BSI Assay
	Sterile fluids and tissues	IRIDICA BAC SFT Assay
	BAL*, ETA**	IRIDICA BAC LRT Assay

KPC, VanA, VanB, mecA

The IRIDICA BAC BSI Assay: Rapid, Sensitive and Culture-Independent Identification of Bacteria and *Candida* in Blood

Metzgar D. *et al.*, PlosOne; July 2016

Johns Hopkins Hospital; Baltimore, USA

285 whole blood samples (with SIRS)

Samples processed by Ibis Bioscience

Limit of detection (LOD) in blood:

S. aureus, 32 CFU/mL; *E. faecium* 16 CFU/mL; *K. pneumoniae* 32 CFU/mL

273 (95.7%) valid results

61 negative controls: all negative by IRIDICA

Organisms detected: IRIDICA, n=85; culture, n=45

11 of these 46 pathogens gave subsequent infection

Species ID

	Matched Positive	BAC BSI Assay + / Culture –	BAC BSI Assay –/ Culture +	Matched Negative[B]
Gram-positive (including Mycoplasma)	15	11[A(5)]	2	207
Gram-negative	13	21[A(4)]	3	207
Unidentified bacteria	0	1	0	207
Yeast	2	1	0	207
Potential Contaminants (details in Table 4)	2	12[A(2)]	3	207
Other reportable organisms excluding potential contaminants (n = 550)	0	0	0	207

32 out of 40 by culture (80% agreement)
Excluding contaminants 30/35 (86% agreement)

Antibiotic R markers

	Matched Positive	BAC BSI Assay + / Culture –	BAC BSI Assay –/ Culture +	Matched Negative
bla_{KPC} (carbapenem resistance[A])	0	0	0	0
vanA/vanB (vancomycin resistance[A])	0	0	0	4
mecA (methicillin resistance[A])	6	0	0	3

13/13

Broad-Range Detection of Microorganisms Directly from Bronchoalveolar Lavage Specimens by PCR/Electrospray Ionization-Mass Spectrometry

Ullberg M. *et al.*, PlosOne; Jan 2017

Karolinska University Hospital, Sweden

121 BAL samples

May 2014 – March 2015

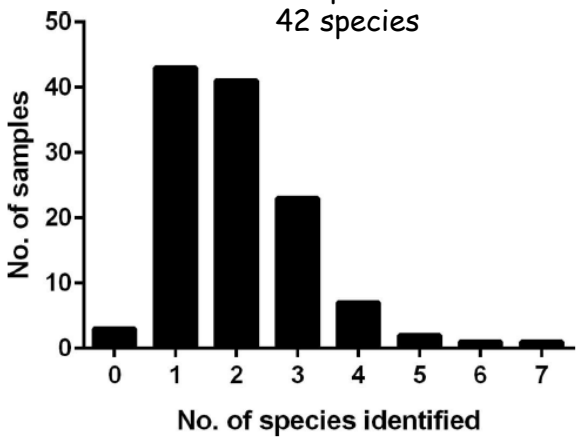
Routine: 104+/121 (86%)

IRIDICA: 118+/121 (98%)

245 organisms:

18 species

42 species



Pathogenic species

	IRIDICA-positive	
	Total	Confirmed by culture and/or PCR
Gram-positive bacteria		
Staphylococcus aureus	33	27[a]
Streptococcus pneumoniae	17	6
Corynebacterium pseudodiphtheriticum	1	-
Gram-negative bacteria		
Haemophilus influenzae	20	16
Klebsiella pneumoniae	4	4
Enterobacter cloacae-complex	5[b]	4
Escherichia coli	2[c]	2
Escherichia vulneris	1	-
Proteus mirabilis	2	1
Proteus vulgaris	1	-
Klebsiella oxytoca	1	-
Pseudomonas aeruginosa	8	7
Stenotrophomonas maltophilia	2	1
Chryseobacterium indologenes	1	-
Atypical/ fastidious species[d]		
Nocardia species	1	1
Bordetella pertussis	1	1
Legionella pneumophila	1	1
Mycoplasma pneumoniae	1	1

15 by routine culture
2 by routine culture

Not detected by routine culture (but other methods)

Detection of resistance determinants

- *mecA* in 21/121 (17%), but only 8/21 were also positive for *S. aureus*
- MRSA not detected by culture and only 1 *S. aureus* had growth
- No BAL with *vanA/B* or bla_{KPC}

} Mostly CoNS+

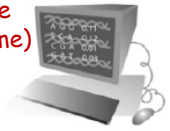
Identification of bacterial pathogens and antimicrobial resistance directly from clinical urines by nanopore-based metagenomic sequencing Schmidt K. *et al.*, JAC; 72: 2017

Norfolk/Norwich Hospitals, UK
10 urines (>10^7 CFU/mL) and 5 spiked (MDR-*Ec*)
MinION: rapid, low capital cost, small

Sample preparation → 105 min → DNA extraction → 30 min → Library preparation → 15 min → MinION sequencing → 60 min → Bioinformatic analysis → 30 min → Results
Bacterial & Resistance genes I/D


4-10 mL urine


7x coverage (99% genome)
500 Euro/cell



Initially, BLAST and CARD databases
WIMP Metrichor application } 7-8 hrs
Metrichor's ARMA } (~4 hrs)

Species ID

	CU5	CU6	Urine spiked with *E. coli* from CU 6	CU7	CU8	CU9	CU10	Urine spiked with *E. coli* H141480453 run 1	Urine spiked with *E. coli* H141480453 run 2	Urine spiked with *E. coli* H141480453 run 3
Best species match to MinION sequence data	*K. pneumoniae* CG43	*E. coli* JJ1886	*E. coli* JJ1886	*E. coli* PMV-1	*E. coli* 536	*E. cloacae* NCTC 9394	*K. pneumoniae* CG43	*E. coli* APEC O78	*E. coli* K-12	*E. coli* APEC O78
Best species match to Illumina sequence data	*K. pneumoniae* MGH 78578	*E. coli* JJ1886	*E. coli* JJ1886	*E. coli* IHE3034	*E. coli* 536	*E. cloacae* NCTC 9394	*K. pneumoniae* CG43	*E. coli* ST410	*E. coli* ST410	*E. coli* ST410

In all cases, **MinION correctly identified the pathogen** (WIMP in 15 min)

ARGs (*vs.* MICs/EUCAST and Illumina HiSeq)

Urine and species	Method[a]	AMP	AMC	TZP	CTX	CTX/CLO	CTX/CLA	CAZ	CAZ/CLA	FEP	FEP/CLA	FOX	AZT	CIP	AMK	TOB	GEN	STR	TMP
CU9 *E. cloacae*	MICs	>64	64	4	2	≤0.12	2	1	1	≤0.12	0.12	>64	0.25	≤0.12	1	0.5	0.5	S	S
	MinION							*ampC* ($bla_{CMY\ mv}$, $bla_{ACT-18,\ 24}$)											
	Illumina							*ampC* (bla_{ACT-24})											
CU10 *K. pneumoniae*	MICs	>64	32	>64	>256	256	0.125	128	1	64	≤0.06	16	>64	>8	8	>32	>32	R	R
	MinION	$bla_{TEM\ (mv)}$, bla_{OXA-1}						$bla_{CTX-M\ gr1}$, $bla_{SHV(mv)}$						*aac(6')-Ib-cr*, *qnrB*	*aac(6')-Ib-cr*, *aacA4*, *aacC2*, *aadA3*, *strA*, *strB*				*dfrA14*
	Illumina	bla_{TEM-1}, bla_{OXA-1}						$bla_{CTX-M-15}$, bla_{SHV-28}, bla_{LEN-12}						*gyrA* (83:S-I), *parC* (80:S-I), *aac(6')-Ib-cr*, *qnrB*	*aac(6')-Ib-cr*, *aacC2*, *strA*, *strB*				*dfrA14*

Illumina detected 55 ARGs from cultured strains. MinION detected 51 of them.

But, **allelic variants poorly distinguished** (e.g., mutations in *gyrA/parC*; cAmpCs and pAmpCs)

Conclusions

Species identification →
- Some systems do not provide species ID
  For rectal swabs ID probably not necessary
- Only some report many species [Verigene, FilmArray, Unyvero, Iridica, MinION]

Resistance mechanisms →
- Most systems have a limited list of ARGs (e.g., only KPC)
- Only some report many ARGs [Verigene, Unyvero, MinION]
- Inability to detect new/emerging ARGs
- Inability to recognize variants (e.g., cAmpCs *vs.* pAmpCs)
- Is the detected ARG expressed?
- Only two link the ARG with the species ID [Xpert, MinION]

Kit and automated →
- Almost all are relatively easy to use (from samples to results)

High sensitivity/specificity →
- In general they have good sensitivity (but LOD < than culture)
- Specificity is low for non-sterile specimens (e.g., from LRT)
  Difficult interpretation (what will we tell to clinicians?)

Clinical samples →
- Most systems are used for rectal swabs or positive BCs
- Only IRIDICA for whole blood
- "Not ready" for direct implementation in respiratory samples

Rapid (TAT) →
- From <1 h [colorim., Xpert, FilmArray, Eazyplex] to 4-8 h [Unyvero, Iridica, MinION]
- The overall outcome is affected in positive (but lack of studies)

Cost-effective →
- From ~10 Euro [e.g., colorimetric] to ~600 Euro [e.g., MinION]
- Who pay? Still difficult to convince institutions

THANK YOU!

Institute for Infectious Diseases - University of Bern, Switzerland

- Dr. Valentina Donà, PhD (PostDoc)
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- João Pires (PhD student)
- Regula Tinguely (Lab Tec)
- Sara Kasraian (Lab Tech)

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